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REGULAR ARTICLE

Microorganisms and nematodes increase levels of secondary metabolites in roots and root exudates of *Plantago lanceolata*

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Abstract Plant secondary metabolites play an important role in constitutive and inducible direct defense of plants against their natural enemies. While induction of defense by aboveground pathogens and herbivores is well-studied, induction by belowground organisms is less explored. Here, we examine whether soil microorganisms and nematodes can induce changes in levels of the secondary metabolites aucubin and catalpol (iridoid glycosides, IG) in roots and root exudates of two full-sib families of *Plantago lanceolata* originating from lines selected for low and high constitutive levels of IG in leaves. Addition of soil microorganisms enhanced the shoot and root biomass, and the concentration of aucubin in roots of both *Plantago* lines without affecting IG levels in the rhizosphere. By contrast, nematode addition tended to reduce the root biomass and enhanced the stalk biomass, and increased the levels of aucubin and catalpol in root exudates of both *Plantago* lines, without affecting root IG concentrations. The *Plantago* lines did not differ in constitutive levels of aucubin and total IG in roots, while the concentration of catalpol was slightly higher in roots of plants originally selected for low constitutive levels of IG in leaves. Root exudates of "high IG line" plants contained significantly higher levels of aucubin, which might be explained by their higher root biomass. We conclude that soil microorganisms can induce an increase of aucubin concentrations in the roots, whereas nematodes (probably plant feeders) lead to an enhancement of aucubin and catalpol levels in root exudates of *P. lanceolata*. A potential involvement of secondary metabolites in belowground interactions between plants and soil organisms is discussed.

Keywords Belowground defense · Iridoid glycosides · Root exudates · Nematodes · Soil microorganisms

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Introduction

Induction of plant defense has mainly been studied aboveground (e.g. Karban and Baldwin 1997). However, plants also interact with numerous organisms, including pathogens and herbivores, below ground. Only few studies have considered the induction of

plant defenses in roots. Studies with root-feeding insects show induction of higher levels of secondary metabolites in roots (Birch et al. 1992; Bezemer et al. 2003, 2004; Borowicz et al. 2003; van Dam and Raaijmakers 2006). In addition to induction of direct defenses, induction of indirect defenses, i.e. the recruitment of antagonists of the attacker by the plant, might also occur belowground (Boff et al. 2001; van Tol et al. 2001). Indirect defenses belowground might be mediated through the attraction of the antagonists by belowground plant chemicals (Rasmann et al. 2005; Rasmann and Turlings 2008). However, whether these chemicals are actually released from the roots into the rhizosphere is often unknown, as they have mostly been extracted from the root tissue and not from, for example, root exudates. Therefore, in our study we tested for induction of secondary plant metabolites in both root tissue and root exudates by soil organisms.

Plantago lanceolata (ribwort plantain), is a plant species with a world-wide distribution that serves as a model plant species in many biotic interaction studies (e.g. Bowers 1983; Bowers and Puttick 1988; Marak et al. 2002a, b). Iridoid glycosides (IG) are important secondary metabolites of *P. lanceolata* that deter aboveground generalist insect herbivores (Bowers and Puttick 1988) and pathogens (Marak et al. 2002a), whereas they may act as feeding and oviposition stimulants for specialist herbivores (Bowers 1983) and affect the performance of their parasitoids (Harvey et al. 2005). Aboveground herbivores (Darrow and Bowers 1999) and pathogens (Marak et al. 2002b) can induce the production of IG. A recent study (Wurst et al. 2008) also documented an induction of the IG catalpol in roots after herbivory by wireworms. As far as we are aware, no study has been published investigating the induction of IG in both roots and root exudates when *P. lanceolata* is challenged by soil microorganisms and nematodes.

Here we investigated whether secondary metabolites in roots and root exudates of a plant can be induced by soil microorganisms and nematodes using *P. lanceolata* as model plant. We conducted a greenhouse study with two different full-sib families of *P. lanceolata* from lines originally selected for low and high constitutive levels of IG in leaves (Marak et al. 2000). The plants were grown in sterilized field soil or in sterilized soil re-inoculated with either addition of the soil microorganism community (within a soil suspension <20 μm) or the nematode community (both from a field site where *P. lanceolata* occurs naturally) and their combination. We chose the whole soil microbial and nematode community rather than selected species to investigate the overall effect of the natural communities on the plants. Besides documenting whether the full-sib families from genetically different plant lines of *P. lanceolata* differ in biomass, nutrient contents, and levels of IG in roots and root exudates, we tested the hypothesis that soil microorganisms and nematodes induce changes in the levels of secondary metabolites in roots and in root exudates.

Materials and methods

Seeds of two full-sib families of *Plantago lanceolata* from lines selected for low and high constitutive leaf iridoid glycoside levels (Marak et al. 2000) were surface sterilized with sodium hypochlorite (1%), sown on wet paper in Petri dishes and placed in the greenhouse (16 h light, 20°/25°C night/day temperature). Germinated plants were transplanted into seedling trays filled with gamma-sterilized (25 kGray) soil 1 week after sowing.

A loamy, sandy mineral soil (N=0.13%, C=2.1%, C/N=16.7) from Mossel (52°04′N, 05°45′E, The Netherlands) was used in the experiment. The soil was sieved and gamma-sterilized (25 kGray) and 140 kg (fresh wt) was equally divided between two plastic boxes of 40 cm height, 60 cm length and 40 cm width, that had been surface sterilized with ethanol (90%). One box with 70 kg of soil received 700 ml of a soil microorganism suspension made from randomly collected sub samples of 100 g non-sterilized experimental soil, originating from the same field site, suspended in 1 l demineralized H_2O and filtered through 1×75, 3×45 and 1×20 μm sieves ("soil microorganisms" treatment) (Wurst et al. 2009). The other box of 70 kg received 700 ml demineralized water to create a non-inoculated "sterilized" soil. The boxes were closed with a plastic top cover and left to incubate for ca. 3 weeks, opened twice a week for 1 h to expose the soil to microorganisms from the greenhouse environment and mixed thoroughly to homogenise for effects of the random microbial colonization of the soils that cannot be avoided in greenhouse studies lasting for several months.

Nematodes were extracted from randomly collected 24 kg of non-sterilized soil from the same field site. The nematodes were extracted by decantation (Oostenbrink 1960), which involves a 3 times repetition of suspending 1.5 kg soil in 4 l water, then pouring the suspension over 1×75 and 3×45 μm filters. The debris from the filters was collected, put on paper filter (Hygia favoriet, 220 mm; NIPA Instruments, Leeuwarden, NL) and incubated at 20°C for 48 h. After the nematode extraction by decanting, nematodes were identified and counted using a microscope (magnification 10×20). In 1 ml of the suspension on average of 329 nematodes were present consisting of 64.2% bacterial feeders, 19.0% omni- and carnivores and 16.8% root and hyphal feeders.

Experimental set-up

To establish a full factorial experiment with the soil organism treatments "soil microorganisms" and "nematodes", 48 pots (11.5 cm height, 13 cm diameter) were set up in a greenhouse under 16 h day length and 20°/25°C night/day temperature conditions. Half the pots (N=24) were filled with 950 g of the sterilized soil; the other 24 pots received the "soil microorganisms" soil. Seedlings were planted from seedling trays into the pots. The pots were planted with one individual of *P. lanceolata*; half the pots were planted with a seedling from the "low IG line" and the other pots were planted with a seedling from the "high IG line". After 4 days, half the experimental pots of each pre-treatment received 10 ml of the nematodes suspension divided over 5 ca. 2-cm deep holes at a distance of 1–2 cm from the plant; the other half received the same amount of tap water. There were 6 replicates of each treatment and the pots were redistributed randomly every week to avoid position effects. The pots were watered regularly and brought to the same moisture content (0.32 vol/vol) every week. In week 11 of the experiment, root exudates potentially containing IG were obtained by sampling sterile soil solutions from the rhizosphere with polyethersulfone (PES) micro-suction cups (Micro-rhizon, Frits Meijboom, Wageningen, NL) of a pore size <0.2 μm (Shen and Hoffland 2007). First, the pots were brought to higher water content (0.44 vol/vol) and then the micro-suction cups were inserted at a distance of 1 cm from the basal stem to a depth of around 5 cm into the rhizosphere of the plant. A sterile 10 ml syringe provided an under-pressure and the sampling took place for 1.5 h; then, after adding another 20 ml of demineralised water to the pots, the sampling went on for another 30 min. The samples (between ca. 500 and 1,000 μl were obtained) were collected in 2 ml Eppendorf cups and frozen at −20°C. In week 12 of the experiment, the plants were harvested. First, the aboveground plant parts were cut off and separated into leaves and stalks (= whole inflorescence). Soil cores were taken for the nematode extraction (approximately 100 g fresh wt). Roots were washed, the shoot and roots were frozen at −80°C and afterwards freeze-dried.

Chemical analyses

The freeze-dried leaf and root samples were ground to a powder and the concentrations of N and C were measured with a C/N analyzer (Flashea Series 1112, Interscience, Breda, NL). Iridoid glycosides (IG) from 25 mg freeze-dried and ground root samples were extracted overnight with 70% methanol. The concentrations of the IG aucubin and catalpol were analysed using a Dionex (Sunnyvale, CA) HPLC (BioLC) equipped with a GP50 gradient pump, a Carbopac PA1 guard (2×50 mm) and analytical column (2×250 mm), and an ED50 elektrochemical detector for pulsed amperimetric detection (PAD). NaOH (1 M) and ultrapure water were used as eluents (10:90%). Retention times were 3.5 min and 5.0 min for aucubin and catalpol respectively. Concentrations were analysed using Chromeleon Software Release 6.60 (Dionex Corp.). The same HPLC method was used to analyze IG from the untreated root exudates samples collected with the micro-suction cups.

Soil organisms

The experiment described here was a subset of a bigger experiment that included also other plant species (see Wurst et al. 2009). The fungal communities of the inocula and from the rhizosphere of all plant species including *P. lanceolata* treated with and without soil microorganism and nematode addition were characterized using PCR-DGGE as described in Wurst et al. (2009). The microbial fungal communities differed significantly between the inocula ("soil microorganisms" vs. "nematodes") and remained distinct until the end of the experiment (data not shown, but see Wurst et al. 2009).

The nematodes were extracted from 100 g of fresh soil and from roots of the soil cores (Oostenbrink 1960) and identified to feeding type at the end of the experiment and counted under a microscope (magnification 10×20).

Statistical analysis

Data were analysed by three-way factorial analyses of variance (ANOVA) in a general linear model (GLM, Statistica 6.0, Statsoft) with the categorical factors "plant line", "soil microorganisms" and "nematodes". The data were tested for normality (Kolmogorov-Smirnov one-sample test) and homogeneity of variances (Levene test) and log-transformed or arcsine transformed (for percentage data) if necessary.

Results

Soil organisms

The nematodes at the end of the experiment showed different abundances according to the soil organism treatments in the rhizospheres of the plant lines. Bacterial feeders reached similar numbers in both the soil microorganism and nematode treatments and their numbers were higher under "high IG" plants ($F_{[1,24]}$= 4.39, P<0.05). Nematode addition treatments had significantly more plant and fungi feeders ($F_{[1,24]}$= 8.78, P<0.01) and omni- and carnivores ($F_{[1,24]}$= 23.65, P<0.001) compared with the treatments without nematode addition. Plant line had no significant effect on the numbers of plant and fungi feeders ($F_{[1,24]}$=1.54, P>0.05) and omni- and carnivores ($F_{[1,24]}$=2.43, P>0.05; Table 1).

Plant biomass

The root biomass of both *P. lanceolata* lines tended to be reduced when nematodes were added (Table 2, P= 0.051, Fig. 1), while the added soil microbial community enhanced the root biomass of both plant lines (Table 2, Fig. 1). Without addition of nematodes, the enhancement of root biomass by soil microorganisms seemed to be stronger in plants from the "high IG line" than in plants from the "low IG line" (Fig. 1), but this was not reflected in a significant interaction between plant line and soil microorganism treatment (Table 2). Overall, the "high IG line" plants produced more root biomass than the "low IG line" plants (Table 2, Fig. 1). Nematode addition did not affect leaf biomass, but nematodes enhanced stalk (= inflorescence) weight of both *P. lanceolata* lines (Table 1). The "low IG line" plants had a higher stalk weight than the "high IG line" plants (Table 1, Fig. 2). The addition of the soil microorganisms also increased leaf biomass (Table 1, Fig. 1), resulting in a greater total biomass ($F_{[1,40]}$=17.02, P<0.001). The "high IG line" plants had a lower shoot to root ratio than the "low IG line" plants (Table 2). Nematode addition had a marginally significant positive effect on shoot to root ratio (Table 2).

Plant nutrient content

N and C concentrations in roots did not differ between the plant lines and were not affected by the addition of soil microorganisms or nematodes (Table 2 and Table 3). However, addition of nematodes increased leaf N concentration in plants from the "high IG line", whereas it decreased that in plants from the "low IG line" (Interaction between plant line

Table 1 Effects of the addition of soil nematodes and microorganisms on the numbers of nematodes of different feeding classes in 100 g soil at the end of the experiment. B = Bacterial feeders, P + F = plant and fungi feeders, O + C = omnivores and carnivores. Means (SE)

	Plantago low IG			Plantago high IG		
	B	P + F	O + C	B	P + F	O + C
Control	0 (0)	1 (1)	1 (1)	0 (0)	0 (0)	0 (0)
Microorganisms (M)	704 (132)	1 (1)	0 (0)	1,059 (347)	1 (1)	0 (0)
Nematodes (N)	536 (155)	4 (2)	20 (12)	910 (142)	19 (14)	4 (2)
N + M	723 (287)	4 (2)	23 (11)	1,178 (234)	13 (6)	10 (2)

Table 2 ANOVA table on the effects of plant line and soil organism treatments on plant biomass (g dry wt), root chemistry (% dry wt) and rhizosphere iridoid glycoside content (μg/ml rhizsophere solution, log transformed)

Source	df	Root biomass		Leaf biomass		Stalk biomas		Shoot/Root	
		F	P	F	P	F	P	F	P
P: Plant line	1	**5.16**	**0.029**	**19.92**	**<0.001**	**8.96**	**0.005**	**13.42**	**0.001**
M: Microorganisms	1	**8.86**	**<0.001**	**12.31**	**0.001**	1.49	0.229	2.48	0.123
N: Nematodes	1	4.06	0.051	0.57	0.455	**5.19**	**0.028**	3.91	0.055
PxM	1	2.39	0.130	0.09	0.766	3.05	0.088	1.52	0.225
PxN	1	0.34	0.563	0.28	0.601	0.03	0.873	0.10	0.759
MxN	1	0.06	0.815	1.13	0.294	1.27	0.267	0.36	0.549
PxMxN	1	2.65	0.111	0.00	0.952	2.02	0.163	1.85	0.181
Error	40								
Source	df	Root nitrogen		Root carbon		Root aucubin		Root catalpol	
		F	P	F	P	F	P	F	P
P: Plant line	1	0.06	0.809	0.40	0.551	0.00	0.981	**10.85**	**0.002**
M: Microorganisms	1	2.40	0.129	0.10	0.704	**19.95**	**<0.001**	3.65	0.065
N: Nematodes	1	0.77	0.386	0.50	0.482	0.02	0.878	0.79	0.380
PxM	1	0.05	0.817	0.00	0.837	0.29	0.596	0.14	0.707
PxN	1	0.22	0.640	0.10	0.787	0.07	0.791	0.00	0.948
MxN	1	0.73	0.399	0.40	0.526	2.14	0.153	1.07	0.310
PxMxN	1	0.76	0.388	0.00	0.826	1.95	0.172	0.36	0.554
Error	40					32			
Source	df	Root IG		Rhiz. aucubin		Rhiz. catalpol		Rhiz. IG	
		F	P	F	P	F	P	F	P
P: Plant line	1	1.47	0.235	**6.75**	**0.013**	1.73	0.196	**4.86**	**0.033**
M: Microorganisms	1	**18.31**	**<0.001**	1.12	0.297	0.25	0.619	0.83	0.369
N: Nematodes	1	0.20	0.658	2.63	0.113	1.59	0.215	2.42	0.128
PxM	1	0.32	0.574	0.94	0.337	0.21	0.646	0.69	0.412
PxN	1	0.04	0.850	1.16	0.288	0.99	0.326	1.16	0.288
MxN	1	2.40	0.131	0.13	0.723	0.01	0.925	0.10	0.759
PxMxN	1	0.82	0.372	0.18	0.671	0.11	0.738	0.17	0.680
Error	32			39					

and nematodes: $F_{[1,40]}=5.88$, $P<0.05$; Table 3). The C concentration in leaves of the "high IG line" plants was higher than in plants from the "low IG line" ($F_{[1,40]}=7.54$, $P<0.01$; Table 3).

Secondary metabolites in roots

Total IG and aucubin concentrations in the roots did not differ between the plant lines, but the catalpol concentration in roots was lower in plants from the "high IG line" than in plants from the "low IG line" (Table 2, Fig. 3b). Addition of soil microorganisms enhanced the aucubin concentration in the roots of both *P. lanceolata* lines (Table 2, Fig. 3a). Catalpol showed the same tendency, but the difference was not significant (Table 2, Fig. 3b). Nematode addition did not affect the IG concentrations in the roots of *P. lanceolata*.

Secondary metabolites in root exudates

Aucubin and catalpol were detected in the root exudates of both *P. lanceolata* IG lines. Plants from the "high IG line" contained higher aucubin levels in

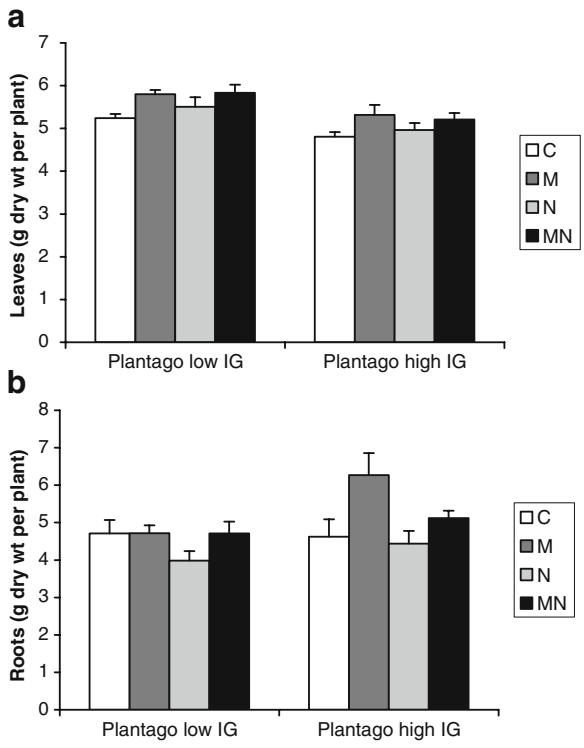

Fig. 1 Effects of soil microorganisms (M) and nematodes (N) and their combination (MN) on the biomass of **a** leaves and **b** roots of two *Plantago lanceolata* lines (Plantago low IG and Plantago high IG). Means + SE. For ANOVA results see Table 1

root exudates than plants from the "low IG line" (Table 2, Fig. 4), but this effect of plant line disappeared when root biomass was considered as a covariate (Table 4). The ANCOVA revealed that iridoid glycoside levels in root exudates increased with root biomass, and that nematode addition significantly enhanced IG levels in root exudates of *P. lanceolata*, irrespective of the plant line (Table 4, Fig. 5)

Discussion

The two full-sib families from lines selected for low and high IG levels in leaves (Marak et al. 2000) differed only slightly in their belowground IG contents. Catalpol concentrations in roots of plants from the "low IG line" were higher than the levels in the roots of the plants from the "high IG line", whereas aucubin levels were not different between the two IG lines. This is in line with observations based on 86 full-sib families from these selection lines (de Deyn et al. 2009) showing that despite a general positive genetic correlation between root and shoot levels of IG, there is quite some variation in this relationship, i.e. not all families with high shoot IG levels also have high root IG levels. While aucubin levels in roots did not differ between the plant lines, more aucubin was detected in the rhizosphere of plants from the "high IG line", which could be explained by their higher root biomass, since the effect disappeared when root biomass was included as covariate.

The inoculated soil microorganisms induced the production of aucubin in roots and also tended to increase the levels of catalpol in roots. In a former study, increased aucubin levels were detected in leaves of *P. lanceolata* when an organic litter patch was present in the rhizosphere (Wurst et al. 2004), suggesting that the observed induction might have been due to enhanced microbial activity in the litter patch. Studies focusing on aboveground interactions of *P. lanceolata* with microorganisms reported an induction of both aucubin and catalpol in leaves and roots after infection of the stalks by a fungal pathogen (Marak et al. 2002b). These previous and present results suggest that there is an involvement of aucubin in interactions of *P. lanceolata* with microorganisms, both above and below the ground. More research is needed to elucidate the factual role of root secondary metabolites in plant defense below the ground.

Addition of the nematode community, which included plant feeders, did not affect IG levels in

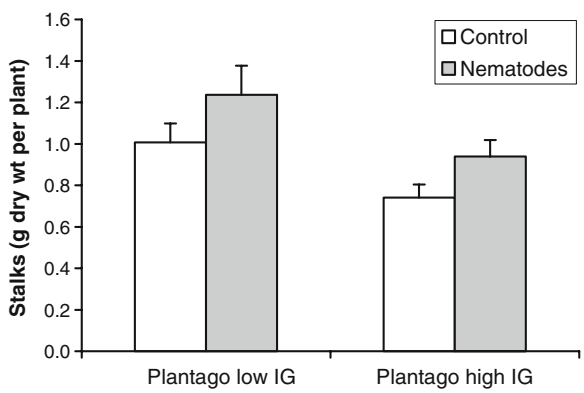

Fig. 2 Effects of nematodes on the stalk biomass of two *Plantago lanceolata* lines (Plantago low IG and Plantago high IG). Combined means + SE. For ANOVA results see Table 1

Table 3 Effects of the soil organism treatments on the N and C concentration (% dry wt) in leaves and roots of the plants. Means (SE)

	Plantago low IG		Plantago high IG	
Leaves	**N**	**C**	**N**	**C**
Control	0.69 (0.03)	44.09 (0.23)	0.81 (0.03)	45.30 (0.34)
Microorganisms (M)	0.85 (0.06)	44.63 (0.52)	0.75 (0.04)	44.70 (0.44)
Nematodes (N)	0.67 (0.03)	44.48 (0.44)	0.82 (0.06)	45.28 (0.38)
M + N	0.67 (0.03)	44.08 (0.30)	0.85 (0.06)	44.97 (0.33)
Roots	**N**	**C**	**N**	**C**
Control	0.44 (0.02)	42.29 (0.34)	0.43 (0.01)	42.19(0.49)
Microorganisms (M)	0.44 (0.02)	42.16 (0.31)	0.44 (0.02)	41.86 (0.25)
Nematodes (N)	0.42 (0.01)	42.30 (0.37)	0.44 (0.02)	42.22 (0.10)
M + N	0.47 (0.02)	42.35 (0.18)	0.46 (0.01)	42.28 (0.33)

roots. Likewise, an earlier study showed that inoculation with the endoparasitic nematode *Pratylenchus penetrans* did not affect IG levels in the leaves of *P. lanceolata* (Wurst and van der Putten 2007). Therefore, nematodes do not appear to affect IG levels in roots of *P. lanceolata*, neither when exposing the plants to a mixed community, nor to a specific root-feeding nematode species. By contrast, root-feeding insect larvae are known to induce IG in this plant species, at least in the roots. Wireworms induced catalpol in roots (Wurst et al. 2008), but not in leaves of *P. lanceolata* (Wurst and van der Putten 2007). More studies are needed to see whether the induction of IG in roots generally differs between nematodes and insect root herbivores and whether the feeding mode and the degree of specialisation play a role. In contrast to the lack of effect on IG in roots, the levels of both aucubin and catalpol in root exudates were enhanced when the soil nematode community was added. However, it remains unknown whether the exudation of plant secondary metabolites in presence of nematodes is a plant defense response or a by-product of the feeding

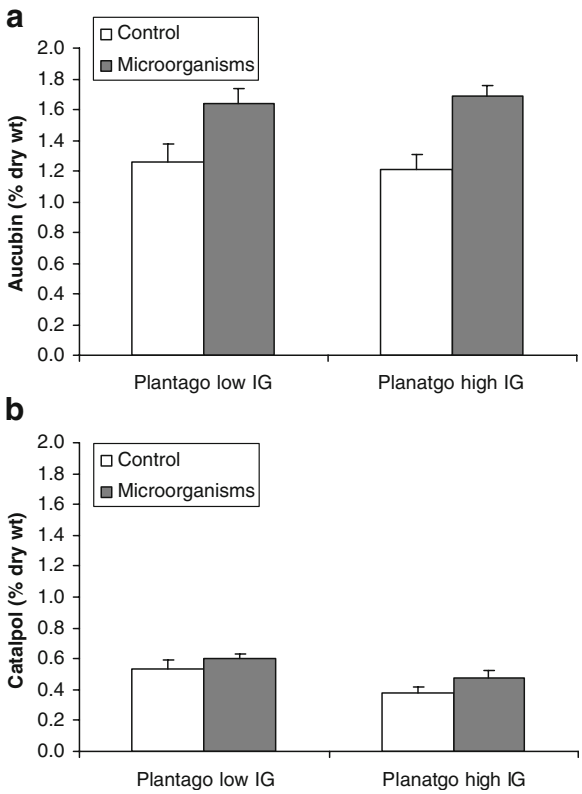

Fig. 3 Effects of soil microorganisms on **a** aucubin and **b** catalpol concentrations in roots of two *Plantago lanceolata* (Plantago low IG and Plantago high IG). Combined means + SE. For ANOVA results see Table 1

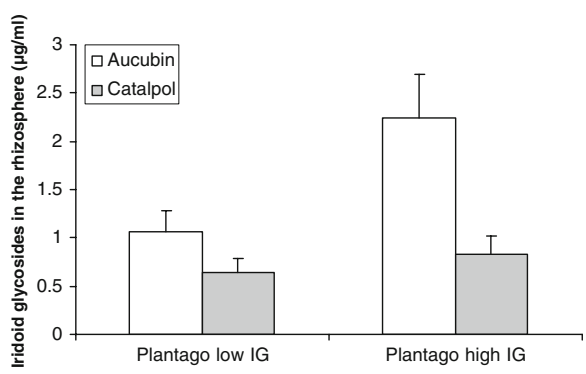

Fig. 4 Effects of plant line (low IG line and high IG line) on the iridoid glycosides (aucubin and catalpol) in the rhizosphere of *Plantago lanceolata*. Combined means + SE. For ANOVA results see text

Table 4 ANCOVA table using root biomass as covariate on the effects of plant line and soil organism treatments on rhizosphere iridoid glycoside content (μg/ml rhizosphere solution, log transformed)

		Rhiz. aucubin		Rhiz. catalpol		Rhiz. IG	
Source	df	F	P	F	P	F	P
Root biomass	1	**7.97**	**0.008**	**8.19**	**0.007**	**8.30**	**0.006**
P: Plant line	1	2.75	0.105	0.12	0.734	1.55	0.220
M: Microorganisms	1	0.01	0.908	0.46	0.501	0.08	0.780
N: Nematodes	1	**6.55**	**0.015**	**4.85**	**0.034**	**6.33**	**0.016**
PxM	1	0.10	0.754	0.05	0.821	0.02	0.878
PxN	1	1.90	0.176	1.68	0.203	1.92	0.173
MxN	1	0.29	0.595	0.06	0.800	0.24	0.628
PxMxN	1	1.23	0.274	1.05	0.312	1.23	0.273
Error	38						

activity of nematodes causing leakages from the roots (Yeates et al. 1999; Haase et al. 2007). A recent study on Arabidopsis (Badri et al. 2008) indicates that the release of phytochemicals by roots is partially controlled by ATP-binding cassette transporters. The latter study also reports big differences between the phytochemical profiles of root tissues and root exudates suggesting a very effective excretion of some compounds that were only found in root exudates.

Nematode addition reduced the root biomass, but led to more investment in stalks of *P. lanceolata*. Since we added a whole nematode community this effect could be either a response to phytophagous nematodes leading to a different plant development rate, or to bacterivorous nematodes releasing nutrients by feeding on bacteria (Ferris et al. 2004). However, since bacterivorous nematodes also established in the soil microorganism treatments, the effects on plant biomass was likely caused by phytophagous nematodes.

Overall, plant biomass was not affected much by the addition of nematodes. In contrast, the soil microbial community enhanced both the root and leaf biomasses. Since the soil microbial inoculum contained no mycorrhizal fungi due to the small pore size (20 μm) of the filter, the growth promoting effects were probably caused by higher numbers of beneficial microorganisms such as plant growth promoting rhizobacteria.

The N and C concentrations in roots were not affected by the addition of soil microorganisms or nematodes, nor the plant line. The C concentration in leaves was higher in the plants from the "high IG line". The N concentration in leaves was affected by an interaction between nematode addition and plant line. Leaf N concentration increased in plants from the "high IG line", but decreased in plants from the "low IG line" when nematodes were added. Since the plant lines differ in a number of physiological traits (Marak et al. 2000) they might also respond differently to biotic agents such as plant-feeding nematodes. This is in line with earlier studies showing that aboveground defense responses of *P. lanceolata* to

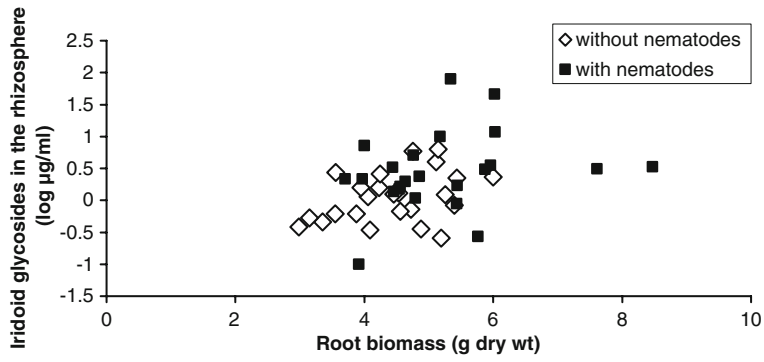

Fig. 5 Effects of root biomass (g dry wt) and nematodes on the iridoid glycosides (aucubin + catalpol) levels (μg/ml, log transformed) in the rhizosphere of *Plantago lanceolata*. For ANCOVA results see Table 3

wireworms differed between plants from the "low IG line" and the "high IG line", leading to different levels of leaf damage by naturally occurring herbivores (Wurst et al. 2008).

In both the soil microorganism and the nematode treatment bacterivorous nematodes reached similar abundances. In a greenhouse environment colonization of open pots by bacterivorous nematodes is common. However, bacterivorous nematodes only established in the pots where soil microorganisms had been added, but not in the sterilized control pots. This indicates either that small eggs of bacterivorous nematodes have passed through the 20 μm sieve or that bacterivorous nematodes from the greenhouse environment could only establish in pots where soil microorganisms had been added and thus a diverse food source was present. As the effects of the soil microorganism as well as the nematode addition treatment persisted despite equal numbers of bacterivorous nematodes in both treatments, we do not think that the bacterivorous nematodes themselves contributed to the treatment effects. The other feeding groups, plant and fungi feeders and omni- and carnivores, were only present in the nematode addition treatment or present in the nematode treatment in significantly higher numbers. This indicates that the effects of the nematode addition treatment were probably due to these feeding groups, and most likely due to plant-feeding nematodes since they directly interact with plant roots. The soil microorganism addition treatment was distinct in its microbial fungal community from the other treatments throughout the experiment (Wurst et al. 2009).

In conclusion, the present study documents that secondary metabolites (IG) are present in root exudates of *P. lanceolata* and are increased by addition of nematodes (in root exudates) and soil microorganisms (in root tissue). Since the role of secondary metabolites in belowground plant defense is far from understood, future studies are needed including studies on the response of soil organisms to plant secondary metabolites and on the mechanisms of excretion of these compounds into the rhizosphere.

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